Single-Molecule Studies of Adenovirus Maturation

Turkin, Alex; Mangel, Walter F.; Oijen, Antoine M. van

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Obtaining Quantitative Parameters of DNA-Ligand Cooperative Binding from Persistence Length Measurements

Livia Siman[1], Ismael S.S. Carrasco[2], Jafferson K.L. da Silva[1], Maria Cristina Oliveira[1], Marcio S. Rocha[2], **Oscar N. Mesquita**[1].
[1]Federal University of Minas Gerais, Belo Horizonte, Brazil, [2]Federal University of Viçosa, Belo Horizonte, Brazil.

Binding of ligands to DNA can be studied by measuring the change of the persistence length of the complex formed, in single-molecule assays. We have measured the persistence length of DNA molecule for cationic and neutral beta-cyclodextrin binding, using optical tweezers. We propose a methodology for persistence length data analysis based on a quenched disorder statistical model and describing the binding isotherm by a Hill-type equation. We obtain an expression for the effective persistence length as a function of total ligand concentration, which fits very well our data of the DNA-cationic beta-cyclodextrin and the DNA-HU protein data available in the literature. The fit returns the values of the local persistence lengths, the dissociation constant and the degree of cooperativity for both sets of data. In both cases the persistence length behaves non-monotonically as a function of total ligand concentration. We discuss some physical mechanisms for these binding processes and their interplay with DNA flexibility.

References
M. S. Rocha *et al*., J. Chem. Phys. **127** (10), 105108 (2007);
J. van Noort *et al*., PNAS **101** (18), 6969 (2004);
D. Sagi *et al*., J. Mol. Biol., **341**, 419 (2004).

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Probability of Double-Strand Breaks in Genome-Sized DNA by γ-Ray Decreases Markedly as the DNA Concentration Increases

Shunsuke Shimobayashi[1], Takafumi Iwaki[2], Toshiaki Mori[3], Kenichi Yoshikawa[4].
[1]Department of Physics, Graduate School of Science, Kyoto University, kyoto, Japan, [2]Fukui Institute for Fundamental Chemistry, kyoto, Japan, [3]Radiation Reserch Center, Osaka Prefecture University, Sakai, Japan, [4]Doshisha university, kyotanabe, Japan.

DNA double-strand breaks (DSBs) present a serious threat to all living things and thus many quantitative studies have been carried out. However, there is no established hypothesis that accounts for the statistics of their production, in particular, the number of DSBs per base pair per unit Gy, P_1, which is the most important parameter for evaluating the degree of risk posed by DSBs. In fact, the reported values scatter by three orders of magnitude [1,2]. This scattering is partly attributable to varying DNA concentrations. So, we evaluate DSBs caused by γ-ray with giant DNA (166 kbp) for a wide region of DNA concentrations using high sensitive single-molecule observation [3]. We find that P_1 is inversely proportional to the concentration above a certain threshold. We give a theoretical interpretation in terms of attack of reactive species upon DNA molecules. Our theoretical model suggests the importance of the size of DNA and its characteristics as semiflexible polymers.

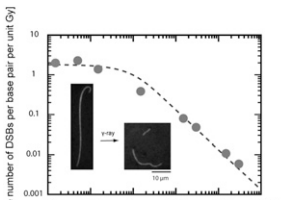

Figure 1: Log-log plot of the number of DSBs per base pair per unit Gy, P_1, as a function of the DNA base-pair concentration, C_{bp}. It is seen that P_1 is roughly constant for small values of C_{bp} and inversely proportional to C_{bp} for large values.

[1] Sutherland B M et al., PNAS **97**: 103-108 (2000).
[2] Chen C-Z et al., Electrophoresis **10**: 318-326 (1989).
[3] Yoshikawa Y et al., Chem. Phys. Lett. **456**: 80-83 (2008).

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Probing Single-Molecule Enzymatic Dynamics of B-Glucosidase using Zero-Mode Waveguides

Ryo Iizuka[1], Ikumi Toshimitsu[1], Kentaro Tahara[1], Hirokatsu Arai[2], Toshihiro Tetsuka[2], Koji Matsuoka[2], Shou Ryu[3], Yuji Asano[3], Takashi Tanii[3], Kiyohiko Igarashi[1], Masahiro Samejima[1], Takashi Funatsu[1,4].
[1]The University of Tokyo, Tokyo, Japan, [2]Saitama University, Saitama, Japan, [3]Waseda University, Tokyo, Japan, [4]JST-CREST, Tokyo, Japan.

β-glucosidases (BGLs, EC 3.2.1.21) are exo-type enzymes that hydrolyze β-glucosidic bonds from the non-reducing end of their substrates. BGLs are present in all living organisms (bacteria, archaea, and eukarya), and perform a range of functions. In bacteria and fungi, BGLs play an important role in cellulose saccharification, which catalyzes the hydrolysis of cellobiose and short cellooligosaccharide to glucose. Due to their potential biotechnological importance, a large number of BGLs from bacteria and fungi have been cloned and characterized. Interestingly, these enzymes often exhibit the enzymatic properties such as substrate and product inhibition. Thus, the dynamic molecular properties are too complicated to be fully understood by the use of conventional ensemble techniques. We then employed a single-molecule assay to probe the enzymatic dynamics of BGL.

BGL1B from the wood-rotting basidiomycete Phanerochaete chrysosporium was used in this study. As the enzyme has a relatively low affinity for cellobiose (Km = ~200 μM), μM concentration of fluorescent cellobiose is required to monitor the enzymatic reaction. Therefore, we employed a single-molecule assay using zero-mode waveguides (ZMWs). ZMWs comprise nanoscale holes in an aluminum film deposited on a fused silica coverslip, and can reduce the observation volume by more than three orders of magnitude relative to conventional microscopic techniques, allowing single-molecule observations at μM concentrations of fluorescent molecules in solution. Biotinylated BGL1B was immobilized in ZMWs through a biotin-streptavidin linkage. The BGL1B was immersed in a solution containing 1 μM tetramethlyrhodamine-conjugated cellobiose (TMR-cellobiose). The repeated appearance and disappearance of TMR fluorescence were often observed, indicating that immobilized BGL1B hydrolyzes TMR-cellobiose in ZMWs. Surprisingly, we have found that the enzymatic reaction is inhibited by glucose non-competitively at the lower concentration and competitively at the higher concentration.

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DNA Dynamics under Nano-Confinement

Tomasz Bakowski, Helmut Strey.
Stony Brook University, Stony Brook, NY, USA.

DNA polymer dynamics are fundamental to the function of biological systems. Examples include gene regulation, cell division, threading and transport through pores. We studied the dynamics of DNA molecules ranging in length from 40bp to 20kbp both in solution and under confinement in nano-slits. DNA molecules were 2-color end labeled and their internal polymer dynamics were observed by fluorescence microscopy and fluorescence cross correlation spectroscopy. We also present a modified fitting model for FCCS results taking into account confocal volume overlap imperfection and nano-slit confinement. We experimentally determined the diffusion coefficients of a range of different weight DNA molecules. Better understanding internal polymer dynamics under nano-confinement has potential applications in genomic sequencing, single biomolecule manipulation, and separations as well as the ability to address fundamental research questions in biophysics and molecular biology.

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Single-Stranded DNA Curtains for Real-Time Single-Molecule Visualization of Protein–Nucleic Acid Interactions

Bryan Gibb[1], Tim D. Silverstein[1], Ilya J. Finkelstein[2], Eric C. Greene[1].
[1]Columbia University, New York, NY, USA, [2]University of Texas, Austin, TX, USA.

Single-molecule techniques have greatly advanced our understanding of many types of biochemical reactions. We have established strategies for anchoring and organizing 'double-stranded' DNA (dsDNA) molecules on the surfaces of microfluidic sample chambers that are coated with a fluid lipid bilayer. This technique called ''DNA Curtains'' has proved powerful in the examination of proteins as they bind, diffuse and translocate along dsDNA. Single-stranded DNA (ssDNA) is a crucial intermediate in nearly all biochemical reactions related to the maintenance of genome integrity. However, most single molecule studies of proteins on ssDNA use short synthetic oligonucleotide substrates. Here, we present procedures for generating, aligning and visualizing hundreds of long single-stranded DNA molecules along the leading edges of nanofabricated barriers, where the DNA can either be ''single-tethered'' or ''double-tethered''. This new approach permits long-desired access to critical biological reactions involving single-stranded DNA binding proteins.

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Single-Molecule Studies of Adenovirus Maturation

Alex Turkin[1], Walter F. Mangel[2], Antoine M. van Oijen[1].
[1]University of Groningen, Groningen, Netherlands, [2]Brookhaven National Laboratory, Uppton, NY, USA.

Instead of relying only on three-dimensional diffusion to associate with a target on DNA, many DNA-binding proteins reduce the dimensionality of search by transiently diffusing along DNA and thus speed up the recognition process. Previously, it has been shown that during the maturation of a single adenovirus particle ~70 copies of the adenovirus protease (AVP) have to cleave ~3200 target proteins situated on the viral DNA in order to render virus particles

infectious. The crowded environment in the 100-nm sized viral particle makes it impossible for the AVP proteins to utilise regular three-dimensional diffusion to find the large number of protease targets. Instead, adenovirus proteins employ a small peptide pVIc as a ''molecular sled'', which allows for one-dimensional sliding along DNA. We use single-molecule techniques to verify the one-dimensional movement model of pVIc peptide and to decipher the molecular mechanisms underlying adenovirus maturation.

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Single Molecule Optical Determination of Bestrophin Stoichiometry

Shashank Bharill, Zhu Fu, Ehud Y. Isacoff.
University of California Berkeley, Berkeley, CA, USA.

Best macular dystrophy (BMD) is an autosomal dominant form of macular degeneration, linked to mutations in the hBEST1 gene, which encodes the calcium activated chloride channel (CACC) bestrophin-1. The bestrophin family includes three additional members: hBEST2, 3 and 4. Because mutations in hBEST1 cause BMD, but a knock-out does not, hBEST1 mutants have been suggested to exert a dominant negative effect through interaction with other CACCs. Using single molecule subunit counting and co-localization we find that each hBEST forms a homotetrameric channel. Despite considerable conservation among hBESTs, hBEST1 has little or no interaction with other hBESTs. Deletion and chimera analysis are being used to identify the portions of hBEST1 that allow assembly of like subunits and prevent assembly with other hBESTs. Our results suggest that the pathology caused by hBEST1 mutations is not due to assembly with other CACCs.

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Incremental Phosphorylation of a Dynamic Lawn of NDC80 Complexes Provides Graded Control of Kinetochore-Microtubule Affinity

Anatoly Zaytsev[1,2], Lynsie J.R. Sundin[3], Boris Nikashin[1], Keith F. DeLuca[4], Jeanne Mick[3], Geoffrey J. Guimaraes[3], Fazly I. Ataullakhanov[1], Ekaterina L. Grishchuk[2], Jennifer G. DeLuca[3].
[1]CTPPhChPh RAS, Moscow, Russian Federation, [2]University of Pennsylvania, Philadelphia, PA, USA, [3]Colorado State University, Fort Collins, CO, USA, [4]Colorado State University, Fort Collins, UT, USA.

The stability of kinetochore-microtubule (KMT) attachments is finely tuned to drive different mitotic processes. This regulation involves phosphorylation of NDC80 complex, a major component of the MT-binding interface at kinetochores. The fundamental question of how the number and stability of KMT attachments is regulated by NDC80 phosphorylation remains unanswered. The Hec1 subunit of the NDC80 complex has an unstructured ''tail,'' which is required for KMT attachment in vivo and contributes to the NDC80 complex-MT binding in vitro. This tail is an established target for Aurora kinases and has 9 mapped phosphorylation sites. To understand how phosphorylation of the Hec1 tail affects MT-binding characteristics of single NDC80 complexes we expressed and purified NDC80Bonsai complexes with different number of phospho-mimetic mutations in Hec1 tail. With TIRF microscopy we found that Hec1 tail phosphorylation leads to a graded increase in NDC80 diffusion and the shortening of its MT residency time, but cooperativity of NDC80-MT binding is only weakly affected by Hec1 tail phosphorylation. To understand physiological relevance of the phosphoregulation of NDC80 complexes we used computational approaches to model kinetochore-MT interface containing multiple NDC80 molecules. We show that the behavior of such interface strongly depends on the spatial organization of the NDC80 complexes. KMT interface that contained ''repetitive sites'' of NDC80 complexes greatly amplified the relatively small, phosphorylation-induced changes in the residency time of single NDC80. However, the KMT interface that contained a dynamic ''lawn'' of un-clustered and uncoordinated NDC80 complexes exhibited a graded response to phosphorylation and produced an excellent fit to our data with cells in prometaphase and metaphase. We conclude that incremental phosphorylation of NDC80 complexes drives the graded regulation of kinetochore-microtubule affinity during mitotic progression.

Biomolecular NMR Spectroscopy

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Solution Structure and Dynamics of a Plant Pathogen Effector

James L. Tolchard[1], Vicki S. Chambers[1], Laurence S. Boutemy[2], Mark J. Banfield[2], **Tharin M.A. Blumenschein**[1].
[1]University of East Anglia, Norwich, United Kingdom, [2]John Innes Centre, Norwich, United Kingdom.

Plant diseases account for at least $220 billion of crop losses worldwide every year, and have a significant impact on global food security and bio-fuel availability. Bacterial, oomycetes and fungi pathogens secrete protein effectors into plant cells, where they perturb cellular processes, presumably to the benefit of the pathogen. In oomycetes, an N-terminal protein motif (RXLR) is important for targeting plant pathogen effectors into host cells (Whisson *et al*. 2007, *Nature***450**, 115 and Oliva *et al*. 2010, *Cell. Microbiol.***12**, 1015). However, little is known about the molecular mechanisms by which RXLR effectors manipulate host cell pathways. We have been studying the RXLR effector AVR3a11 (from *Phytophthora capsici*, a pathogen of pepper), which shares sequence similarity with the well-studied AVR3a effector from the Irish potato famine pathogen *Phytophthora infestans* (the causative agent of late blight in potato and tomato). Using a combination of 2D and 3D nuclear magnetic resonance (NMR) spectroscopy experiments, 75% of the AVR3a11 backbone has been assigned. With additional ^{13}C-HSQC-NOESY and ^{15}N-HSQC-NOESY experiments, a structural ensemble model has been generated which is in good agreement with the X-ray structure of AVR3a11 (PDB code 3ZR8, Boutemy *et al*. 2011, *J. Biol. Chem.***286**, 35834). Backbone amide T_1, T_2 and heteronuclear NOE relaxation experiments at 800 MHz, show that AVR3a11 behaves as expected for a well ordered protein. However, the measured transverse relaxation is faster than theoretically expected for a protein this size, suggesting that conformational exchange may be occurring. Additional information on conformational exchange was obtained from hydrogen/deuterium exchange experiments.

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E. Coli F1Fo ATP Synthase Subunit C: Solution NMR Structure by CS-Rosetta

Yisong Tao, Mark Girvin.
Albert Einstein College of Medicine, Bronx, NY, USA.

The ATP synthase is the main source of ATP in cells, whose function is to inter-convert chemical energy and transmembrane electrochemical potential difference. The enzyme contains two domains: a soluble F_1 domain where ATP is synthesized/hydrolyzed and a transmembrane F_O domain, which functions as an ion pump. Multiple (9-15) copies of subunit c form an oligomeric ring in the F_O domain. The ion translocation activity takes place at the interface of c-ring and subunit a where an essential acidic residue in middle of the outer helix in subunit c undergoes a protonation/deprotonation process, which couples with the relative rotation of c-ring to subunit a. Although high-resolution X-ray structures of the c-rings from several organisms have been solved, the mechanism of ion translocation still needs to be clarified at atomic resolution.

We aim to solve the structures of *E. coli* subunit c at both protonation states of the essential Asp61 and elucidate any conformational changes at the active site during the ion translocation process. Due to the difficulty of obtaining unambiguous long-range NOE constraints in membrane proteins, traditional solution NMR methods were unsuccessful in this case. We employed CS-Rosetta, which utilizes minimal NMR constraints, in our study.

Our study of the subunit c in the protonated state shows that the choice of proper scoring weights is essential in the application of CS-Rosetta to membrane proteins. Using limited NMR restraints this method converges on a structure for *E. coli* subunit c that is similar to the X-ray structures of subunit c from other organisms. Further improving the quality of the structure may be achieved by optimizing the weights in the calculation. We are also using this approach on deprotonated subunit c and hoping to model the c-ring from our results.

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NMR Solution Structure of Opa60: A Neisserial Membrane Protein that Mediates Host Phagocytosis

Daniel A. Fox, Linda Columbus.
University of Virginia, Charlottesville, VA, USA.

The family of Opa proteins from Neisseria gonorrhoeae and N. meningitidis are eight-stranded β-barrel outer membrane proteins that induce human cells to engulf the bacterium by engaging three different host receptors: carcinoembryonic antigen cellular adhesion molecules (CEACAM), heparansulfate proteoglycans (HSPG), or integrins via HSPG and fibronectin or vitronectin. The receptor engaged depends on the sequence of two of the extracellular loops, hypervariable loops 1 and 2, which are highly variable between isolates. Multiple sequence alignment of the HV loops does not reveal specificity motifs among the family of Opa proteins due to the extreme variability in the amino acid sequences. To investigate the determinants of Opa-receptor interactions, the NMR solution structure of Opa60, which engages CEACAM receptors, was determined.

In order to solve the structure, a suite of three dimension NMR experiments were performed to obtain an assignment, each optimized for the different